PB80-185242

The American Oyster ('Crassostrea virginica') as an Indicator of Carcinogens in the Aquatic Environment

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

1979

U.S. Department of Commerce
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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIP
EPA-600/J-79-080	JOURNAL ARTICLE	PB80-185247

4. TITLE AND SUBTITLE	5. REPORT DATE
The American Oyster (Crassostrea virginica) as an Indicator of Carcinogens in the Aquatic Environment	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
John A. Couch, Lee A. Courtney, James T. Winstead and Steven S. Foss	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
SAME AS Box 12	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency Environmental Research Laboratory Sabine Island Gulf Breeze, Florida 32561	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES
Reprinted from 1979
Animals as Monitors of Environmental Pollutants, pp. 65-84, National Acad. Sciences

16. ABSTRACT

The American oyster (C. virginica) was used as the experimental animal for chronic exposure to 3-methylcholanthrene (3-MC) and benzo(a)pyrene (BP) in an exposure system in which the carcinogens can be continuously injected into free flowing water at fixed rates ranging from 1 to 5 µg/l. Experiments designed to determine uptake and distribution of ^{14}C-MC and ^{14}C-BP showed that these are concentrated in oyster tissues in direct proportion to the dosage of carcinogen injected into the system. Residual concentrations as high as 84.4 µg/kg of BP were present in oysters as long as 6 months following exposure. Autoradiography showed intense localization of ^{14}C-BP in distal portions of the tubules of the digestive gland and to a lesser extent in the gonadal tissues. Aryl hydrocarbon hydroxylase (AHH) activity was present in homogenates of hepatopancreas after 5.5 months of exposure to the carcinogens, in contrast to control animals in which AHH activity was quite low. In eight oysters exposed to MC, an infiltration of cells believed to be of hematopoietic origin was encountered in the mantle. Some appear to be identical in feral oyster populations. However, it would be premature at this stage to assign any etiological significance to the experimental findings.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Organic compounds Aquatic animals Pollutants Marine Biology Mollusks Radioautography Benzo(a)pyrene	Oysters Chronic Exposure Aryl hydrocarbon hydroxylase (AHH) activity Carcinogens Crassostrea virginica Benzo(a)pyrene 3-Methylcholanthrene	06/F 06/T 06/S 06/F

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Unlimited	unclassified	
	20. SECURITY CLASS *(This page)* unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
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SPRINGFIELD, VA. 22161

EPA-600/J-79-080
JOURNAL ARTICLE

Reprinted from:
Animals as Monitors of Environmental Pollutants
ISBN 0-309-02871-X
National Academy of Sciences
Washington, D.C. 1979

The American Oyster (*Crassostrea virginica*) as an Indicator of Carcinogens in the Aquatic Environment

JOHN A. COUCH, LEE A. COURTNEY, JAMES T. WINSTEAD, and STEVEN S. FOSS

Synthetic organic chemicals, agricultural chemicals, heavy metals, and petroleum enter the environment as pollutants generated by human activity. Some oncologists now believe that most human neoplasms are related to the presence of carcinogens in the environment (Cairns, 1975). Certain pollutants are actual or potential carcinogens, which may contact aquatic organisms that will accumulate, metabolize and translocate the pollutant or its metabolites (Kraybill, 1975). Results of several studies on the effects, bioaccumulation, and fate of pollutant chemicals in aquatic species have been published (Pimentel, 1971). One potentially serious, but neglected, aspect is the long-term tissue and cellular response of aquatic species that may reflect or indicate the presence of pollutants that are frank or suspect carcinogens.

Evidence that aquatic animal species are susceptible to neoplasia is abundant. However, the etiologies of spontaneous neoplasms in feral aquatic animals are unknown, and the significance of the neoplasms to ecosystems and human health is uncertain (Dawe, 1969). Because some proliferative cellular diseases in fishes and molluscs have been epizootic (Scarpelli and Rosenfield, 1976), the experimental determinations of their etiological agents are necessary. Possible candidate etiologies are chemical pollutants, viruses, and genetic factors.

The selection of aquatic species for use as laboratory carcinogen test indicators is difficult. Many aquatic species are not adaptable or acclimatable to long-term laboratory exposures and manipulations. Aspects of nutritional requirements, behavior, natural disease, life cycle, and hydrographics limit the number of species amenable even

to flowing-water aquarium systems, much less to the static aquarium conditions that are necessary in many inland or metropolitan research centers.

Table 1 lists some aquatic species that are considered suitable for laboratory experimentation. Each of these species has one or more or all of the following qualities that make it a probable candidate for carcinogenesis studies: It can be maintained for relatively long periods under laboratory conditions; it can be manipulated experimentally; it can be reared from egg to adult in the laboratory; it has a history of spontaneous or experimentally induced neoplasia; it is representative of a broad distribution; and it has shown metaplastic, anaplastic, or hyperplastic tissue responses to chemical exposures.

Methods for studying effects of carcinogens in aquatic species must be carefully planned to derive maximum information. Short- or long-term exposures of aquatic organisms to carcinogens require safe and adequate holding facilities (properly isolated and ventilated raceways, aquaria, ponds, or glassware) that insure a stable, healthy background for select test species. There are methods of applying chemicals to exposure water that ensure continual direct or intermittent contact of test chemical with test animals.

In static systems, the chemical is dissolved in solvent or directly in water to attain desired concentration. In flow-through systems, concentrations of stock solution are introduced by controlled injection[1] into systems with known water flow rate to maintain desired ambient concentrations of toxicants. Food, somatic injection, or implant can also be used as vehicles for desired doses of chemical.

The experimental exposure regimens for aquatic species given in Figure 1 (A–D) cover several of the contemporary hypotheses for chemical carcinogenesis. Figure 1A represents long-term continuous exposure of test species to sublethal concentrations of chemical. This method accounts for situations in which subjects are under chronic chemical insult, much as would be the case in low-level, chronically polluted rivers or estuaries. The regimen in Figure 1B could be used to simulate a situation in which an aquatic community receives a single dose of chemical, as in accidental spills of oil or industrial wastes. In Figure 1C aquatic species might receive repeated but discrete exposures to a chemical in order to reproduce regular periodic ocean dumping of municipal or industrial wastes or seasonal pesticide spraying. Figure 1D accounts for a theory of chemical carcinogenesis (Berenblum, 1970)

[1] Several injection-devices are commercially available. These devices use gear-driven plungers in injection syringes. Delivery diluters can be built.

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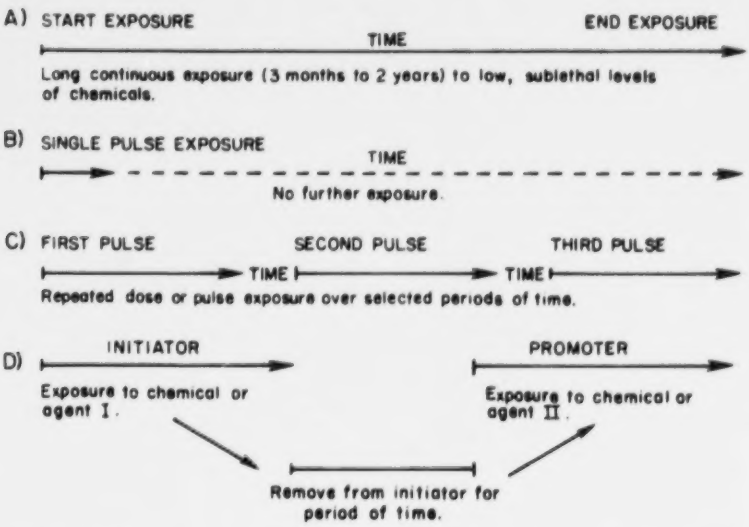

FIGURE 1 Some possible experimental exposure regimens of aquatic species to test chemicals for carcinogenic activity.

that involves an initiating agent followed by a promoting agent. Under aquatic conditions, this sequence could occur if exposure to pollutant with initiating carcinogenic qualities (e.g., a mutagenic substance) was followed by exposure to a second pollutant with tumor-promoting qualities (see Prehn, 1971).

This paper describes the basis for, and the potential laboratory use of, the American oyster (*Crassostrea virginica*) as an indicator of carcinogens in the aquatic environment. The oyster was selected because it fulfills most of the above-listed requirements for an aquatic species to be used in carcinogen studies and because we are familiar with pathology of oysters.

MATERIALS AND METHODS

To date, we have designed and implemented an experimental exposure system for determining the rates of uptake, tissue accumulation, metabolism, and effects of two known mammalian carcinogens in relatively large numbers of oysters. The system consists of four tables that receive flowing estuarine water (10‰ to 25‰ salinity). The

TABLE 1 Aquatic Species Considered Suitable for Carcinogenesis Experiments. Many of These Species Are North American Forms; However, Comparable Species Are Available in Europe, the Soviet Union, and the Orient

Species	Container[a]	Sample Reference on History of Tumor Studies in Species
Freshwater		
Guppy (*Lebistes reticulatus*)	TS	Pliss and Khudoley, 1972
Zebra danio (*Brachydanio rerio*)	TS	Stanton, 1969
Platyfish (*Xiphophorus maculatus*)	TS	Anders, 1967
Swordtail hybrids (*X. helleri*)	TS	Anders, 1967
Rainbow trout (*Salmo gairdner*)	RW, TFT	Halver and Mitchell, 1967
Brook trout (*Salvelinus fontinalis*)	RW, TFT	Wellings, 1969
Yellow bullhead (*Ictalurus natalis*)	TFT, TS	Harshbarger and Couch, in preparation
Channel catfish (*I. punctatus*)	TFT, TS	None
Blue gill (*Lepomis macrochirus*)	TFT, TS	None
Mussel (*Anodonta* sp.)	TFT	Pauley, 1969
Snail (*Biomphalaria glabrata*)	TS	Richards, 1973
Crayfish (*Procambarus clarkii*)	TS, TFT	Harshbarger *et al.*, 1971
Flatworms (*Dugesisa* sp.)	TS, FBS	Foster, 1969
Hydra (*Hydra littoralis*)	TS, FBS	Lenhoff *et al.*, 1969
Marine		
Sheepshead minnow (*Cyprinodon variegatus*)	TFT	None
Croakers, several species	TFT	Harshbarger, 1972, 1974
Dover sole (*Microstomus pacificus*)	TFT	Mearns and Sherwood, 1974
Mosquito fish (*Gambusia affinis*)	TFT, TS	None
Oysters (*Crassostrea* sp.; *Ostrea* sp.)	TFT	Couch, 1969; Farley, 1969
Soft clam (*Mya arenaria*)	TFT	Barry and Yevich, 1972
Octopus (*Octopus vulgaris*)	TFT	Rungger *et al.*, 1971
Cuttlefish (*Sepia* sp.)	TFT	Jacquemain *et al.*, 1947
Shrimp (*Palaemonetes* sp.)	TFT, TS	None

[a] TS = Tank or aquaria, static water, but oxygenated or aerated.
RW = Raceways.
TFT = Tank or aquaria with flow-through water supply.
FBS = Finger bowl, static water, possibly oxygenated or aerated.

water is pumped from Santa Rosa Sound near Pensacola, Fla., travels through an overhead delivery trough, and is siphoned to each water table. Carcinogen-contaminated water was delivered via overflow stand pipes (from carcinogen-receiving water tables) to an effluent-holding pond (Figure 2) and water from the control table was returned to Santa Rosa Sound.

Approximately 1,500 oysters were taken from Apalachicola Bay and acclimated to the holding tanks. A base sample of 150 was examined

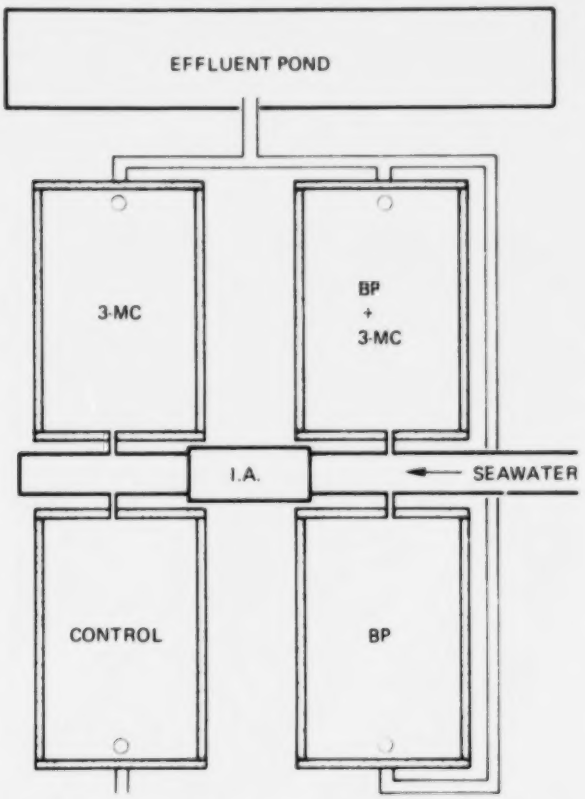

FIGURE 2 Scheme of control and exposure water tables for oysters. BP = benzo[*a*]pyrene exposure; 3-MC = 3-methylcholanthrene exposure; BP + 3-MC = combination exposure; I.A. = injection apparatus.

histologically. Benzo[*a*]pyrene (BP) and 3-methylcholanthrene (3-MC) dissolved in triethylene glycol were each injected into the delivery water of separate tables each containing 300 oysters (Figure 3). In a combination exposure, BP and 3-MC were both injected into the water entering a third table, which also contained 300 oysters. The fourth table, the control table, also containing 300 oysters, received only triethylene glycol in estuarine water. Exposures were made according to a pulse or intermittent schedule to simulate regimen C in Figure 1. The test carcinogens were injected into the system 5 days a week, 8 h a day. The nominal exposure concentration was set at 1 μg of carcinogen per liter of water (1.0 μg/l) from July 14, 1976, to March 22, 1977. At this time the nominal concentration was increased to 5.0 μg/l and exposures continued at this concentration until September 8, 1977, at which time exposure was stopped. Surviving oysters were maintained and depurated in flowing seawater and observed for cellular changes. During the

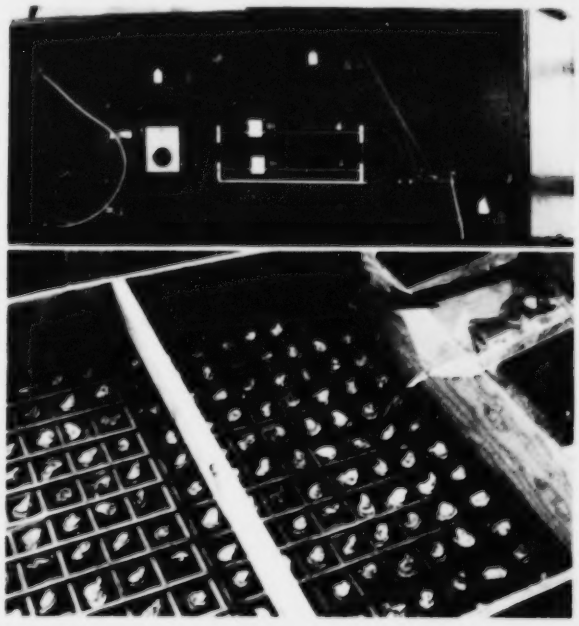

FIGURE 3 Injection apparatus and water table with oysters; carcinogen is injected directly into influent estuarine water which flows into mixing baffle (arrow).

exposure, oysters were able to obtain food from the natural estuarine water delivered to their holding tables. Flow rate of water in each table (700 l/h) was sufficient to support new shell growth in the oysters for the entire test period.

Exposed and control oysters were examined weekly for mortality, growth, and fouling. During the course of the test, a total of seven samples were taken for chemical and histopathological analysis, including a base sample prior to exposure and a depuration sample 3 months after exposure had been terminated. Twelve oysters were taken from each table, for a total of 48 oysters per sample period.

Oyster tissues (1-cm cross section through visceral mass) were fixed in Davidson's fixative, paraffin processed, sectioned at 7 μm, and stained with Harris hematoxylin and eosin. These oyster sections were studied with light microscopy. The remaining tissues from each sample were extracted and analyzed with high-pressure liquid chromatography for the parent compound of polycyclic aromatic hydrocarbons used. Oysters naturally growing in the effluent stream in the receiving pond were analyzed for BP and 3-MC concentrations after 8 months of exposure. Individual oysters after 5.5 months of exposure to BP and 3-MC and controls were analyzed for BP hydroxylase activity by Anderson (1978).

Another experiment to determine rates of uptake and distribution of BP and 3-MC in different tissues of oysters was completed using radiolabeled BP and 3-MC. Two cleaned oysters were placed in each of three 32-l glass aquaria. To one aquarium was added 3 μg/l of ^{14}C-BP (SA 51.0 μCi/μmol) dissolved in a benzene–ethyl alcohol solution (1:50); to a second was added 3.0 μg/l ^{14}C-3-MC (SA 60.2 μCi/μmol) dissolved in benzene–ethyl alcohol solution; to a third was added only the benzene–ethyl alcohol solution in volume equal to that used in the first and second aquaria. To a fourth aquarium, without oysters, 3.0 μg/l ^{14}C-BP in benzene–ethyl alcohol was added. One oyster was taken from each aquarium after 7 days of exposure. Scintillation counts were made on oyster visceral mass samples to determine degree of radioactivity and, hence, degree of accumulation of radiolabeled carcinogen. The remaining oysters from each aquarium were taken after 14 days of exposure and radioactivity was measured. One oyster exposed to ^{14}C-BP for 7 days was allowed to depurate in clean estuarine water for 7 days. Then radioactivity was determined in that oyster. Water samples were taken periodically from the exposure aquaria and the aquarium without oysters to determine rates of loss of labeled carcinogen from water with and without oysters. Histological sections (7 μm) were taken from the visceral masses of each oyster exposed to ^{14}C-BP prior

to scintillation counting and prepared for autoradiography according to the liquid emulsion coating technique of Lillie (1965). Emulsions were exposed for 3, 5, and 7 weeks, developed, and examined for comparative distribution of labeled BP in tissues.

RESULTS

Concentration of Carcinogens in Oysters Exposed Intermittently for 14 Months

Chemical analyses data on uptake and accumulation of BP and 3-MC in oysters are presented in Figure 4. The relatively heavy concentrations of both compounds found in the oysters from the September 1976 sample (193-h exposure) can be explained by the occurrence of an accidental injection at the beginning of the test (July 14, 1976) of relatively high concentrations of carcinogen stock solutions. This occurred when the injection apparatus malfunctioned, resulting in a much faster rate of injection than planned. Subsequent to correcting this malfunction, the desired nominal rate of injection (1 μg/l) was maintained until March 22, 1977 (Figure 4).

In the December 1976 sample (640-h exposure), there were no carcinogens in the oysters analyzed. This complete loss in the second sample of carcinogen from tissues is difficult to explain. There are three probable explanations:

1. Heavy growth of winter algae in exposure tables may have resulted in rapid adsorption and perhaps metabolism of carcinogen by algae, resulting in a lowering of levels of carcinogen available to the oysters.
2. Oyster microsomal enzymes may have been induced and the carcinogens metabolically eliminated (we analyzed only for parent compound, not metabolites); the high levels detected subsequently may be due to the higher levels of carcinogen administered at that time.
3. Errors were made in the chemical analyses of this sample (replicate samples gave the same results).

The next sample, which was taken in March 1977 (1,229-h exposure), revealed carcinogens in all exposed oysters (Figure 4). However, the concentrations did not show a trend of rapid increase. There was only a relatively low increase in the BP-exposed oysters. These oysters had accumulated 209 times the ambient exposure concentration after the December 1976 sampling. Oysters exposed to BP and 3-MC combined showed a tremendous loss of both carcinogens.

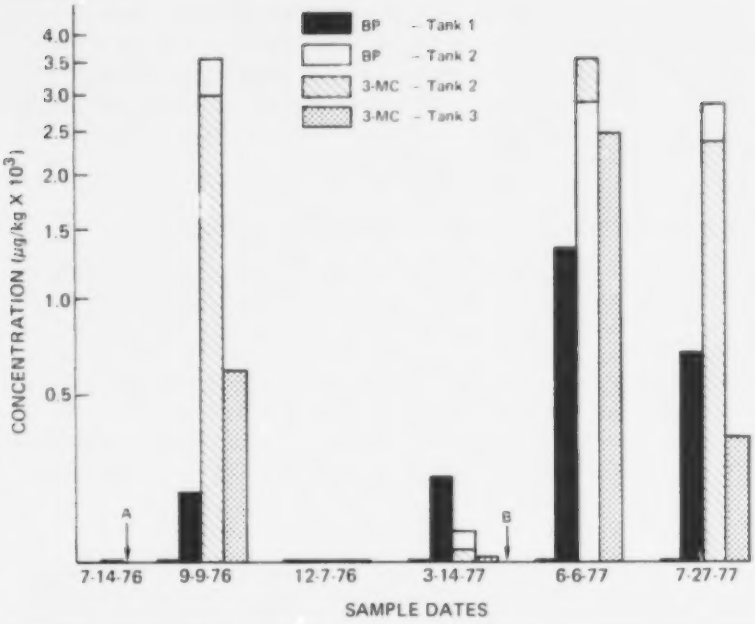

FIGURE 4 Uptake of BP and 3-MC by oysters over 12 months of exposure. Combined open and lined histograms represent results from combination-exposure water table. Point A represents time of accidental "spill" into water tables at beginning of tests. Point B represents time at which concentrations of PAH's were increased from 1 μg/l to 5 μg/l.

Following the March sample, ambient exposure concentration was increased to 5 μg/l (on March 22, 1977). Subsequent samples (June and July 1977) showed corresponding increase in tissue concentrations (Figure 4). Exposure was terminated on September 8, 1977, and a final sample taken in December 1977, after 3 months of depuration, revealed no trace of the parent compounds in any of the test organisms.

Oysters taken from the effluent pond after 6 months of exposure to the mixed effluent had from 13.1 μg/kg to 84.4 μg/kg 3-MC and from a trace to 36.4 μg/kg BP. Those oysters taken nearest the effluent stream had the highest concentrations.

There was aryl hydrocarbon hydroxylase (AHH) activity in hepatopancreas homogenates of oysters exposed 5.5 months to BP or 3-MC. Enzymatic activity was not detected in nonexposed animals (Anderson, 1978). Activity in homogenates from BP-exposed animals was 0.18 nmol BP metabolites per milligram of protein per 10 min.

Similar activity in 3-MC-exposed oysters was 0.21. These data suggest that BP hydroxylase activity may be induced by polycyclic aromatic hydrogen compounds in oysters, as it is in other higher animals. AHH activity is low in oysters compared to that of mammals, but can be consistently shown *in vitro* by the generation of radiolabeled metabolites derived from ^{14}C-BP (Anderson, 1978).

HISTOPATHOLOGICAL EFFECTS OF CARCINOGENS

Oysters in all four water tables had significant mortality in August, September, and October 1976 due to relatively heavy infections by *Dermocystidium marinum*, a common, natural protistan pathogen of oysters of the Gulf of Mexico and Atlantic coast of the United States. Approximately 30% of all oysters in the test was lost during this period. Histological examination determined the extent and nature of the protistan lesions in individual moribund oysters. Because of our extensive experience with natural oyster diseases, we can discriminate

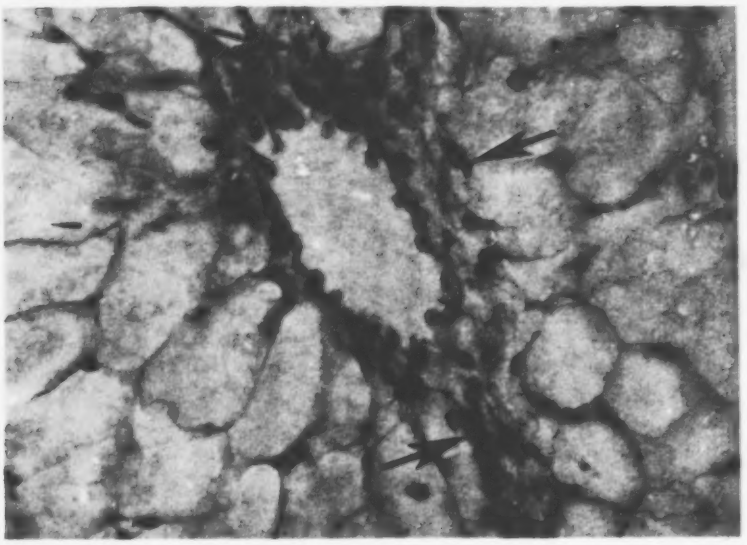

FIGURE 5 Normal blood vessel in mantle of control oyster; note relatively few leukocytes associated with endothelium and in blood sinuses between vesicular connective tissue cells (arrows). (×430.)

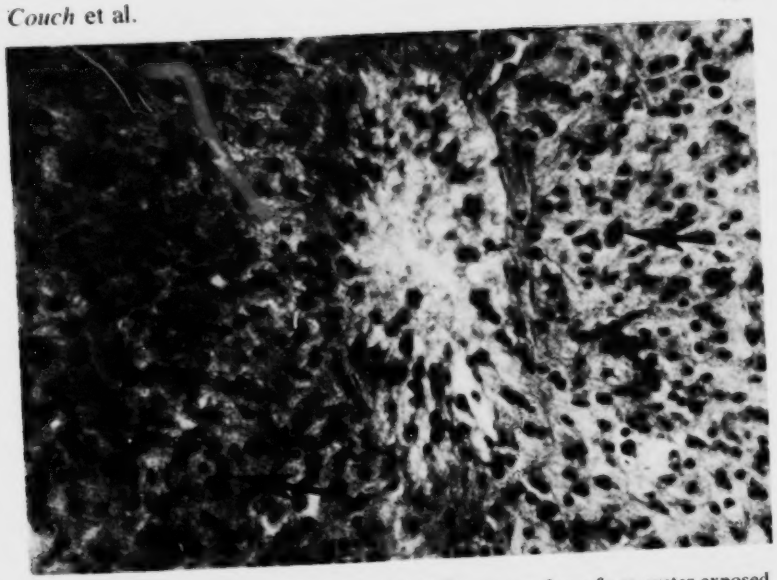

FIGURE 6 Perivascular lesion that resembles inflammatory focus from oyster exposed to 1 μg/l of 3-methylcholanthrene for 1,229 h; note pleomorphic nuclei of cells surrounding blood vessel (arrows). (×430.)

between histological effects of the carcinogens and those of most natural agents on oysters in our region.

Samples of the September 1976 (193-h exposure) and the December 1976 (640-h exposure) oysters exhibited no histopathological changes that could be attributed to the experimental exposures. In these and control animals the tissues were normal (Figure 5). However, in eight of 12 oysters from the 3-MC-exposure (1,229 h of intermittent exposure to 1.0 μg/l 3-MC), there was found a lesion suggesting possible chemical effects. The lesion consisted of incipient perivascular inflammatory foci in the mantle (Figures 6 and 7). Large numbers of at least two different blood cell types made up the lesion surrounding and radiating from the larger blood vessels of the respiratory and somatic mantle. Occasional mitotic figures were found in relatively large cells (nuclear diameter = 3.5 to 6 μm) that predominated in the lesions (Figure 7). This cell type closely resembled those cells constituting the pathologic cell type in sarcomoid neoplasms in feral oysters and clams described by Couch (1969), Farley (1969), and Barry and Yevich (1972) (compare Figures 6, 7 and 8). Both cell types

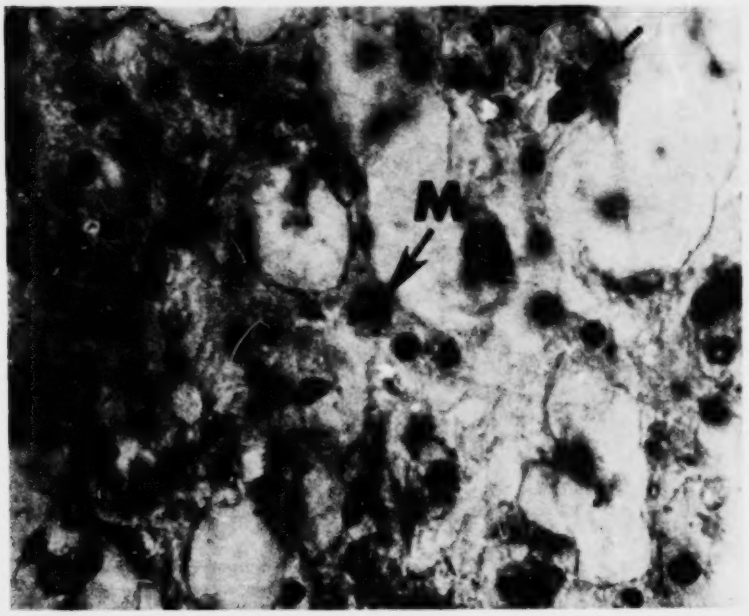

FIGURE 7 Higher magnification of periphery of lesion similar to that illustrated in Figure 6; note mitotic figure (metaphase arrow-M) and other similar nondividing cells (arrows). (×1,000.)

possess pleomorphic nuclei, mitotic activity, and large size relative to normal oyster leukocytes. The extent of lesions in the 3-MC-exposed oysters was not as great as those in the feral molluscan neoplasms. It would be premature, therefore, to consider these lesions in the 3-MC-exposed oysters as unequivocal neoplasms. Further study of other surviving oysters that were exposed to 3-MC will be necessary before the real nature of the perivascular lesions is known.

Accumulation and Tissue Distribution of ^{14}C Benzo[a]Pyrene and ^{14}C 3-Methylcholanthrene

Scintillation count analyses of tissues from oysters exposed for 7 or 14 days to labeled carcinogens in aquaria are given in Table 2. Oysters exposed for 336 h (14 days) to 3.0 μg/l of ^{14}C-labeled compounds

accumulated less or only slightly more carcinogen than oysters exposed for only 168 h (7 days). Concentration factors (concentration in tissues/initial concentration in aquarium water) for different oysters ranged from 204× to 264× in 7- to 14-day exposures to 3.0 μg/l of each carcinogen (Table 2).

Comparison of loss of radioactive label in water samples from aquaria with oysters and without oysters showed a more rapid loss in the aquarium with oysters (lower line in Figure 9) than in the aquarium without oysters (upper line in Figure 9). However, the rate of loss leveled off in both types of aquaria approximately 100 hours after introduction of the compound into the system.

Data on depuration of oysters exposed 7 days then depurated in clean water for 7 days show a 66.8% loss of activity in tissue samples (Table 2).

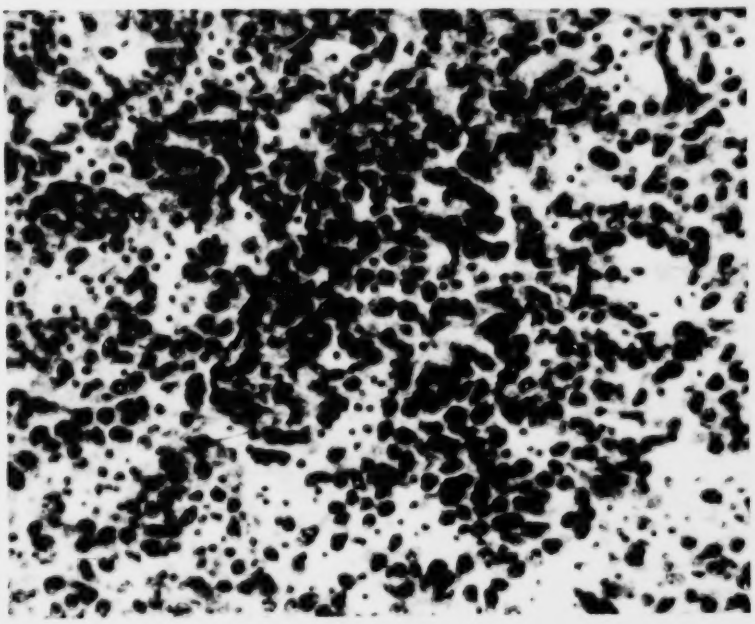

FIGURE 8 Center of neoplastic lesion from a feral oyster suffering from sarcoma-like disease; note cell type morphology and mitotic activity (arrow). (×650.)

TABLE 2 Concentration of ^{14}C Carcinogens in Oysters

^{14}C-Labeled Compound	Exposure at 3 μg/l, h	Total PAH[a] in Oyster, μg	Weight of Oyster, μg	Concentration in Oyster, μg/g	Concentration Factor[c] (× = times)
Benzo[*a*]pyrene	168	7.169	9.80	0.732	244 ×
Benzo[*a*]pyrene	336	4.750	6.70	0.709	236 ×
Benzo[*a*]pyrene	336	1.746	2.40	0.727	242 ×
3-Methyl-cholanthrene	168	3.711	6.05	0.613	204 ×
3-Methyl-cholanthrene	336	4.046	5.10	0.793	264 ×
Benzo[*a*]pyrene	168[b]	7.10	1.727	0.243	66.8%[d]

[a] PAH = polycyclic aromatic hydrocarbons.
[b] 168-h exposure followed by 168-h depuration.
[c] Concentration factors were calculated by dividing final concentration of label in oyster tissue by concentration originally put in aquarium (conversion of specific activity of labeled carcinogen to molar quantities was necessary).
[d] % radioactivity lost during depuration.

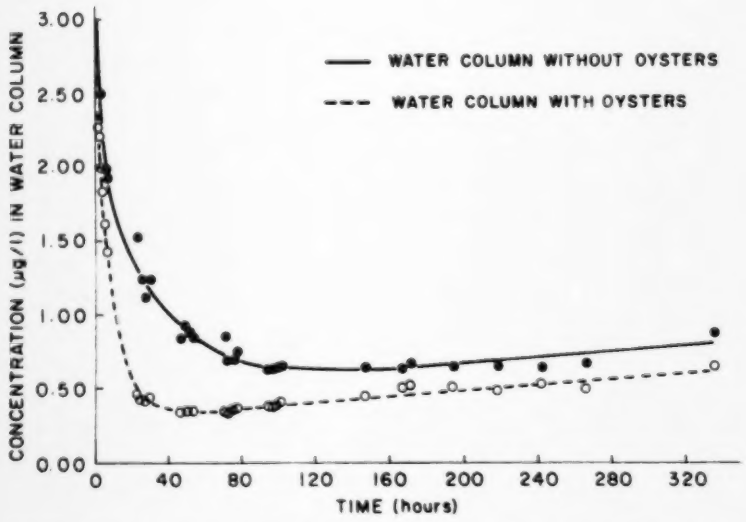

FIGURE 9 Graph of loss of ^{14}C-labeled benzo[*a*]pyrene from water in 32-l aquarium with three oysters (lower curve) and from water in similar aquarium without oysters (upper curve).

These results suggest that oysters take up and accumulate relatively small amounts of BP and 3-MC in contrast to relatively greater uptake of many pesticides (e.g., Parrish *et al.*, 1976; Schimmel *et al.*, 1976). They then quickly either excrete or metabolize (or both) the compounds when returned to clean water.

Radioautographic analyses of tissue sections from the oysters exposed for 7 and 14 days to labeled carcinogens indicate that the distal portions of the digestive tubules in the digestive gland are the chief sites of uptake (Figure 10). Compare this with a histological section from a control oyster shown in Figure 11. Some label occurs in the vesicular connective tissues and gonads surrounding the digestive tubules after 7 to 14 days, but transport to the connective tissue and thence to gonadal tissues probably follows initial absorption by the digestive epithelial cells. Some oyster ova became labeled after 7 to 14 days exposure. Whether or not uptake occurs directly from fluid of the gonoducts or indirectly via the vesicular connective tissue blood sinuses is unknown.

DISCUSSION

The data presented in this report indicate that oysters are capable of concentrating known mammalian carcinogens under long- and short-term experimental exposure regimens. There is some indirect evidence

FIGURE 10 Radioautograph of section of oyster exposed for 7 days to ^{14}C-BP in aquarium; emulsion was exposed for 3 weeks prior to development; note radioactive exposed emulsion and distribution of ^{14}C label as indicated by beta tracks in emulsion (arrows); heavily labeled (DGL) circular areas are epithelial layers of digestive gland tubules. (×4.8.) Go, gonads; I, intestine; G, gills; DGL, digestive gland; S, stomach; M, mantle.

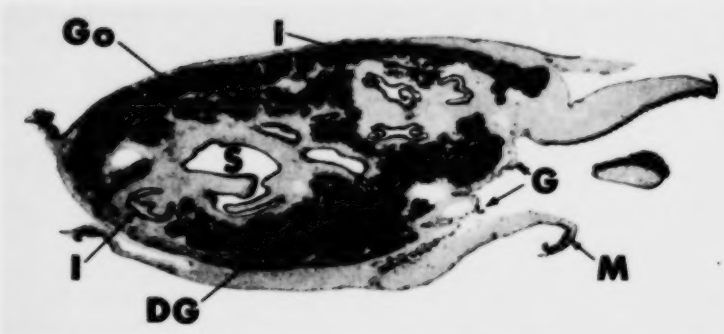

FIGURE 11 Histological section from control oyster for orientation of reader; vesicular connective tissue is light area among circular epithelial layers (arrows). Sections were stained with hematoxylin and eosin. (×4.8.) Go, gonads; I, intestine; G, gills; DG, digestive gland; S, stomach; M, mantle.

from these data and direct evidence from the work of Anderson (1978) that oysters have at least a minimal capacity to metabolize BP. We have not studied the possible role that polycyclic aromatic metabolites may play in oysters. Accumulation of BP and 3-MC by oysters in our studies appeared to be dependent upon the amount of compound available to the oysters, but oysters did not accumulate polycyclic aromatics as rapidly as they have organochlorine pesticides.

Incipient histopathological changes in oysters exposed intermittently to 3-MC for 1,229 actual hours of exposure are suggestive of possible pre-neoplastic or at least inflammatory responses. Survivors of these and longer exposures (14 months) will be examined to determine, if possible, the fate of the 3-MC-related lesions.

Radioautographic studies demonstrated the uptake of both BP and 3-MC by the digestive glands of oysters. No significant uptake was noted for external gill or mantle epithelium. Some ova were heavily labeled with ^{14}C-BP, suggesting that, in the early stages of their life cycles, oysters could be exposed to mutagenic and carcinogenic compounds.

The American oyster *(C. virginica)* appears to be suitable for studies of the fate and possibly the effects of some carcinogens. The fact that oysters can accumulate two known mammalian carcinogens from estuarine waters suggests a need for careful monitoring of these organisms to safeguard human and estuarine species health.

SUMMARY

The American oyster (*C. virginica*) was used as the experimental animal for chronic exposure to 3-methylcholanthrene (3-MC) and benzo[*a*]pyrene (BP) in an exposure system in which the carcinogens can be continuously injected into free flowing water at fixed rates ranging from 1 to 5 μg/l. Experiments designed to determine uptake and distribution of H^3MC and H^3BP showed that these are concentrated in oyster tissues in direct proportion to the dosage of carcinogen injected into the system. Residual concentrations as high as 84.4 μg/kg of MC and 36.4 μg/kg of BP were present in oysters as long as 6 months following exposure. Autoradiography showed intense localization of H^3BP in distal portions of the tubules of the digestive gland and to a lesser extent in the gonadal tissues. Aryl hydrocarbon hydroxylase (AHH) activity was present in homogenates of hepatopancreas after 5.5 months of exposure to the carcinogens, in contrast to control animals in which AHH activity was quite low. In eight oysters exposed to MC, an infiltration of cells believed to be of hematopoietic origin was encountered in the mantle. Some appear to be identical in type to those which constitute sarcoma-like lesions encountered in feral oyster populations. However, it would be premature at this stage to assign any etiological significance to the experimental findings.

ACKNOWLEDGMENT

We thank Dr. Peter Schoor for his help on the chemical analysis performed in his laboratory.

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QUESTIONS AND ANSWERS

M. MIX: I am interested in the curve showing benzo[*a*]pyrene in water alone and in water with oysters. Have you ever conducted studies where you simply put oysters in water and then removed them? We have done this with naphthalene and other compounds, and the curves are remarkably similar for water containing oysters and for water in which oysters were placed briefly and then removed. In other words, we think that there is possibly a substantial amount of bacterial action and not just adsorption to glass and particulate materials.

J. COUCH: I am sure there is even though we clean the oyster shells before placing them in a test situation. Considerable flora and fauna live in the gills, mantle cavity, and in the digestive tract of these animals. These organisms could affect the uptake and metabolism of carcinogens. We have not done what you mentioned, but you should take note that the curve leveled off both with and without oysters in the tanks. This indicates to us that oysters can take the compound out of the water at a certain rate. The oyster is able to control either its accumulation or its metabolism of the compound. At this point, we do not know which. We have repeated this experiment three times and have obtained identical curves. When the curves level off the concentration approaches that in a tank without oysters. Therefore, in a glass-lined aquarium or tank, you are probably seeing the results of an equilibrium between adsorption and desorption on the walls. This is the first study in which I have seen perivascular cellular proliferation of this nature in response to any experimental stimulus (i.e., methylcholanthrene) among the many oysters I have examined in histologic section.

G. MICARI: Dr. Anderson, would you care to comment on the data on benzo[*a*]pyrene hydroxylase activity?

R. ANDERSON: I would like to emphasize the preliminary nature of these data, and also to indicate that one should not assume that the control value is actually zero. We have found subsequently that the method we used to preserve and ship tissues for analysis was not optimal. Apparently the enzyme systems are labile, and a certain amount of activity is lost in rapid freezing. Using the best available method, the activity for aryl hydrocarbon hydroxylase in a normal oyster would be about 0.5 nmol/mg of protein/10 min. It is well established that these enzyme systems are easily induced by polycyclic hydrocarbons (PCB's) and other insecticides in the aquatic environment. Neither the genetic background of these oysters nor their history of exposure to environmental pollutants is known. Consequently, one should expect considerable enzymatic variation among animals due to these variables.

M. LIPSKY: What activation temperature was used in the assays and was there an increase in protein? Also, precisely what fraction was used for the assay, the postmitochondrial supernate, or the pure microsomal fraction?

R. ANDERSON: The optimal *in vitro* temperature is about 37°C, which is considerably higher than that encountered *in vivo*. Apparently the enzyme is

thermostable. By raising the temperature of the incubation mixture one can increase the activity to a point above which it is inactivated. This occurs at 45°C. Enzyme activity can be demonstrated both with whole homogenate and with the microsomal fraction. We did not detect any increase in protein concentration in hepatopancreas preparations of oysters induced by previous exposure to polycyclic aromatic hydrocarbons.

J. WEIS: A bivalve that is subject to heavy pollution is *Modiolus*, the large mussel. Have any neoplasms been described in this species?

J. COUCH: I know of no work on *Modiolus*.

R. BROWN: I have collected and examined a few of the mussels when they were found near *Mya*. Although the numbers are too small to be significant, I did not find any lesions in the animals examined.